The Egyptian Journal of Hospital Medicine (January 2019) Vol. 74 (7), Page 1552-1558

Comparison between The Effect of Sucralose and Sodium Saccharin on Some Physiological Parameters in Male Albino Rats

Eman G.E. Helal[1], Abdullah Al-Shamrani[2], Mohamed A. Abdelaziz[3], Mariam S. El-Gamal[1]
[1]Department of Zoology, Faculty of Science (Girls), Al-Azhar University, [2]Pediatric Pulmonology and Sleep Medicine, Prince Sultan Medical Military City, King Saud bin Abdulaziz University and Health Science,[3]Medical Physiology, Faculty of Medicine, Al-Azhar University
* Corresponding author: Eman Helal, email: emanhelal@hotmail.com, mobile:00201001025364 , orcid.org/0000-0003-0527-7028

ABSTRACT
Background: Non-nutritive sweeteners (NNSs) are becoming popular as sugar substitutes for diabetic patients or to decrease body weight. **Aim of the work:** This study was aimed to determine the effects of sucralose and sodium saccharin on some physiological parameters in male albino rats. **Materials and methods:** We used thirty male albino rats weighing from 100 to120 gm. The period of the experiment was 30 days. The animals were divided into three groups; **Group 1:** control, **Group 2:** rats received sucralose and **group 3:** rats received sodium saccharin. The following parameters were processed: serum glucose, ASAT, ALAT, serum creatinine, serum urea, protein and lipid profiles and hormonal levels (insulin, testosterone, serum T3 and T4). **Results:** There was an increase in ASAT and ALAT activities, serum creatinine and serum urea levels in group 2 and group 3, lipid profile in the group received sucralose (TC, HDL and LDL) and T3&T4 in the group received saccharin as compared to the control group. Meanwhile, a drop in serum glucose, insulin, total protein, albumin, albumin/globulin ratio and triglycerides in group 2 and group 3, lipid profile in the group received saccharin (TC, HDL and LDL) and T3&T4 in the group received sucralose was observed when compared to the control group. **Conclusion:** it could be concluded that sucralose and sodium saccharin must be carefully used because they have very dangerous effects-especially sodium saccharin- and we have to replace them with natural sugar.
Keywords: sucralose, sodium saccharin, ASAT, ALAT, T3,T4, testosterone, insulin.

INTRODUCTION

Artificial sweeteners are used as sugar substitutes called "zero" or "light"- beverages, foodstuffs, pharmaceuticals and personnel care products. They have been used by consumers to acquire a sweet taste, for reasons of economics, blood glucose control, or energy control. The health risks of artificial sweeteners consumption is still a highly controversial topic[(1)]. Artificial sweeteners have allegedly been related to some effects such as cancer, weight gain, metabolic disorders, migraines, type-2 diabetes, vascular events, preterm delivery, kidney function disorders, liver antioxidant system, hepatotoxicity, immune system disruptions and alteration of gut microbiota activity. Human studies failed to show a direct connection to cancer risk. However, other studies, , have shown association with kidney function decline and vascular risk factors[(2)].

Sucralose, one of the newest artificial sweeteners has been approved by the Food and Drug Administration in 1998. Sucralose itself contains no calories but due to its very sweet taste (approximately 600 times as sweet as sugar), sucralose in the granulated format is often mixed with other sweetening ingredients such as maltodextrin. This dilutes its intense sweetness and provides volume and texture[(3)]. It has no adverse effects on the central nervous system, immune system, reproductive performance, and red blood cells constituents and morphology. On the contrary, some reports suggest sucralose is a possible trigger for some migraine patients[(4)].

Saccharin is a non-nutritive, non-caloric intense artificial sweetener. In the European Union, Saccharin is known under the E number E954. It has 300-500 times the sweetness of sucrose, with a slight bitter aftertaste. It is widely used sweetener as it is heat-stable, so, it is used in hot beverages, canned vegetables, bakery products and reduced sugar jams. It has a long shelf life and it is inexpensive. Saccharin goes directly through the human digestive system without being digested; and can trigger the release of insulin in rats[(5)]. Although 66%–84% of saccharin was excreted in the urine and 10%– 40% of the dose was recovered from the feces after dosing for 24 h, traces of saccharin radioactivity remained in many tissues after 72 h, including liver, heart, adrenals, pancreas, thymus, and testes. These data promote the possibility that saccharin may have specific biological functions when entering into these tissues[(6)]. This chemical is one of the most sweeteners that has been extensively studied and investigated due to its possible carcinogenic effects[(7)].

Received:1/12/2018
Accepted:20/12/2018

MATERIALS AND METHODS

Sucralose was obtained as CANDEREL® (Manufactured in EU (Czech Republic) by Merisant Company 2.

Sodium saccharin was obtained as HERMESETAS, Made in Switzerland by Hermes Sweeteners Ltd, Zurich.

Thirty young male albino rats (weighing from 100 to120 g) were used in this study. Animals were housed in stainless steel cages, fed on rat chew and offered water *ad libidum*. The animals were divided into three equal groups (10 rats each) as follows: **The first group:** the control untreated group, **the second group:** rats received orally sucralose (5 mg/kg b.wt./ day) and **the third group:** rats received orally sodium saccharin (5 mg/kg b.wt./day). Body weights were recorded at the beginning and the end. After 30 days, animals were weighed and then decapitated.

Blood samples were collected for biochemical parameters. Blood samples were centrifuged for 15 min. at 5000 rpm and supernatant sera were separated for analysis.

Biochemical Examination

In the present study total protein (TP) and albumin concentration were estimated, then serum globulin concentrations were calculated according to the formula:

Globulin (g/dl) = total protein (g/dl) –albumin (g/dl)

Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) activities, Creatinine, urea, glucose concentrations as well as lipid profile that including total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were also determined. All parameters were estimated using **BioMerieux SA kits, France.**

The ratio of serum albumin/ globulin was determined. However, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were also calculated after calculation of serum LDL-C (low-density lipoprotein cholesterol) and VLDL (very low-density lipoprotein cholesterol) using the **Friedwald's [8]** and **Norbert [9]** formulas, respectively as following:

Friedewald's [8] equation: **LDL (mg/dl) = TC-{HDL + [TG/5]}.**

Norbert [9] equation: VLDL = TG/5

Concentrations of insulin, testosterone and thyroid hormones (T3 and T4) were measured according to the following methods:

An insulin ELISA kit (10-1250-01, Mercodia AB, Uppsala, Sweden) was used to measure insulin levels. **The HOMA-2 IR index** (**Homeostatic Model Assessment of Insulin Resistance**) was calculated by a free online calculator (HOMA Calculator, Version 2.2.3, Diabetes Trail Unit, The University of Oxford, Oxford, UK).

$$\text{HOMA-IR} = \frac{[(\text{Glycaemia}(\text{mg/dl})/18.2) \times \text{Insulin}(\text{mU/ml})]}{22.517} \quad \textbf{(10)}$$

Testosterone, T3 and T4 were estimated by using VIDAS® kits, which is an automated quantitative test.

Statistical analysis

The results were expressed as Mean ± SEM of the mean. Data were analyzed by using T-test and were performed using the Statistical Package (SPSS) program, version 20. The Bonferroni test was used as a method to compare significance between groups.

RESULTS

Body weight: no significant change was noticed in the percentage of body weight change in sucralose group, while there was highly significant decrease ($p<0.01$) in sodium saccharin group as compared to control animals (Table 1).

Glucose level: there was a significant decrease ($p<0.05$) in glucose level in sucralose group and highly significant decrease ($p<0.01$) in sodium saccharin group in contrast to control rats (Table 1).

Insulin level: the present study showed that there was a significant decrease ($p<0.05$) in insulin level of sucralose group, and highly significant decrease ($p<0.01$) in sodium saccharin group comparing to control (Table 1).

HOMA-IR: a significant decrease ($p<0.05$) was observed in the ratio of HOMA-IR in sucralose group, and it was highly significant decrease ($p<0.01$) in sodium saccharin group in comparison to normal rats
(Table 1).

Table (1): Percentage of body weight change, glucose level and HOMA-IR in control, sucralose and sodium saccharin treated animals.

Groups / parameters	Control	Sucralose	Sodium saccharin
% of body weight	8.6±0.5	9±0.6	2±0.8**
% of change		5%	-77%
Glucose (mg/dl)	109±0.7	102.4±1.9*	99±0.7**
% of change		-6%	-9%
Insulin (mU/ml)	0.97±0.007	0.76±0.066*	0.68±0.057**
% of change		-21%	-30%
HOMA-IR	0.26±0.003	0.19±0.021*	0.16±0.009**
% of change		-26%	-38%

Values represent mean ±SE (standard error). (*P**<0.05, *P***<0.01 as compared to control group)

Protein profile: animals that received sucralose has significant decrease (p<0.05) in serum total protein, albumin and albumin/globulin ratio, and those received sodium saccharin showed a highly significant decrease (p<0.01) in the previous parameters as compared to the corresponding control group (Table 2).

Table (2): Serum total protein, albumin, globulin and albumin/globulin ratio in control, sucralose and sodium saccharin treated animals.

Groups / parameters	Control	Sucralose	Sodium saccharin
Total Protein (g/dl)	6.63±0.04	5.69±0.28*	4.2±0.07**
% of change		-10%	-37%
Albumin (g/dl)	3.43±0.046	2.54±0.27*	1.2±0.07**
% of change		-26%	-65%
Globulin (g/dl)	3.2±0.028	3.15±0.08	3±0.09
% of change		-3%	-6%
Albumin/Globulin	1.074±0.02	0.8±0.08*	0.4±0.07**
% of change		-26%	-63%

Values represent mean ±SE (standard error). (*P**<0.05, *P***<0.01 as compared to control group)

Liver enzymes: ASAT and ALAT revealed significant increase (p<0.05) among sucralose group and highly significant increase (p<0.01) among sodium saccharin group in contrast to control rats (Table 3).

Table (3): ASAT and ALAT activities in control, sucralose and sodium saccharin treated animals.

Groups / parameters	Control	Sucralose	Sodium saccharin
ASAT (U/L)	115.88±0.3	122±1.83*	181.6±0.9**
% of change		5%	57%
ALAT (U/L)	55.4±0.16	62±1.98*	83.8±0.86**
% of change		12%	51%

Values represent mean ±SE (standard error). (*P**<0.05, *P***<0.01 as compared to control group)

Lipid profile: the present results revealed highly significant increase (p<0.01) in total cholesterol and HDL-C, significant increase (p<0.05) in LDL-C, highly significant decrease (p<0.01) in triglycerides, and no significant change in VLDL-C and ratios of TC/HDL-C (risk factor 1) and LDL-C/HDL-C (risk factor 2) in rats received sucralose as compared to control group. Meanwhile, there was highly significant decrease (p<0.01) in total cholesterol, triglycerides and HDL-C and significant decrease (p<0.05) in LDL-C but no detectable change in VLDL-C and risk factors in rats received sodium saccharin as compared to the control group (Table 4).

Table (4): Changes in total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C, VLDL-C, TC/HDL ratio and LDL/HDL ratio in control, sucralose and sodium saccharin treated animals.

Groups / parameters	Control	Sucralose	Sodium saccharin
Total Cholesterol (mg/dl)	**121.1±0.38**	**130±0.8****	**105±0.7****
% of change		**7%**	**-13%**
Triglycerides (mg/dl)	**135±0.7**	**128±0.9****	**120±0.8****
% of change		**-5%**	**-11%**
HDL-C (mg/dl)	**59.4±0.48**	**65±0.7****	**51±0.6****
% of change		**9%**	**-14%**
LDL-C (mg/dl)	**34.6±0.48**	**39.4±1.4***	**30±1.3***
% of change		**14%**	**-13%**
VLDL (mg/dl)	**27±0.1**	**25.6±0.8**	**24±1.35**
% of change		**-5%**	**-11%**
TC/HDL	**2.04±0.015**	**2±0.07**	2.058±**0.067**
% of change		**-2%**	**0.8%**
LDL/HDL	**0.6±0.01**	**0.6±0.05**	**0.58±0.02**
% of change		**0%**	**-3%**

Values represent mean ±SE (standard error). (*P**<0.05, *P***<0.01 as compared to control group)

Kidney functions: serum urea and creatinine showed significant increase (p<0.05) in sucralose group and highly significant increase (p<0.01) in sodium saccharin group as compared to control animals (Table 5).

Table (3): Serum urea and creatinine levels in control, sucralose or sodium saccharin treated animals.

Groups / parameters	Control	Sucralose	Sodium saccharin
Urea (mg/dl)	**37.3±0.66**	**41.4±1.1***	**53.4±0.9****
% of change		**11%**	**43%**
Creatinine (mg/dl)	**1.18±0.025**	**1.5±0.098***	**1.9±0.02****
% of change		**27%**	**62%**

Values represent mean ±SE (standard error). (*P**<0.05, *P***<0.01 as compared to control group)

Hormones: sucralose group revealed no detectable change in the level of testosterone hormone, but there was a highly significant decrease (p<0.01) found in its level in sodium saccharin group as compared to control rats. On the other hand, a significant decline (p<0.05) in the levels of both T3& T4 concentrations in sucralose group, but sodium saccharin group showed significant increase (p<0.05) in their concentrations as compared to control values (Table 6).

Table (6): Serum insulin, testosterone, T3 and T4 levels in control, sucralose and sodium saccharin treated animals.

Groups / parameters	Control	Sucralose	Sodium saccharin
Testosterone (ng/dl)	**1.03±0.004**	**0.99±0.03**	**0.65±0.05****
% of change		**-4%**	**-37%**
T3 (ng/dl)	**47.9±0.6**	**43.1±1.38***	**53±1.5***
% of change		**-10%**	**12%**
T4 (µg/dl)	**3.036±0.009**	**2.5±0.16***	**3.5±0.14***
% of change		**-18%**	**15%**

Values represent mean ±SE (standard error). (*P**<0.05, *P***<0.01 as compared to control group)

DISCUSSION

The impact of artificial sweeteners on human health is still a matter of doubtful dispute. The present study revealed that body weight wasn't change by using sucralose. Several studies in rodents announced that sucralose modulates physiological processes involved in nutrient absorption and body weight regulation through its interaction with sweet taste receptors (called T1R2/T1R3) located in enteroendocrine cells of the GIT, pancreatic ß cells, and the hypothalamus[11].

The present results showed highly significant reduction in body weight percent in saccharin group when compared to the control group. In harmony with this result **Dib *et al.*[12]** reported a significant reduction in body weight of rats (50%) after administration of sodium saccharin for 14 days. They attributed this weigh loss to the reduction of food consumption per day as a result of hypotriglyceridemia and hypocholesterolemic effect of sodium saccharin [13].

The administration of sucralose reduced glucose level, as compared to the control. It appears that the decrease of glucose might be resulted from a reduction in its absorption[13], this result was in parallel with **Abou-Donia *et al.*[14]** who reported that the administration of sucralose at 1.1 - 11 mg/kg to male rats for 12-week interferes with the absorption of nutrients and drugs.

In the present work, there was highly significant reduction in blood glucose level of saccharin group as compared to the control group. **Amin *et al.*[5]** have declared that oral administration of low and high doses of saccharin to rats for 30 days induced a decrease in blood glucose, which was in parallel with **Jacquillet *et al.*[15]**. The hypoglycemic effect of saccharin in rats is in accordance with **Abdallah[13]** who noticed that consumption of large amounts of saccharin might reduced blood glucose concentration. This may be due to that saccharin can trigger the release of insulin and thereby reduce blood glucose leve[16].

In our study sucralose reduce insulin secretion. There is a study revealed that sucralose has been indicated to create prediabetic state. The study announced that sucralose ingestion causes significant damage to pancreas leading not only to breakdown in its architecture but also to destruction of its islets and β cells[17].

The study of **Amornpan Lertrit *et al.*[18]** demonstrated reduced acute insulin response (AIR) after a 4-week ingestion of sucralose. This clarify the present results which showed a decrease in HOMA-IR. This may indicate that chronic exposure to sucralose leads firstly to increased insulin secretion, and later to reduction of insulin secretion via depletion of insulin secretorygranules, which leaded to loss of first phase insulin secretion. A defect in early phase insulin secretion is an early predictor of type 2 diabetes mellitus[18].

This study showed a reduction in insulin and HOMA-IR of sodium saccharin group, this is in concomitant with **Bailey *et al.*[19]** who reported a reduction of hyperinsulinemia, decrease the insulin resistance and improve glycemic control during saccharin consumption in hyperglycemic obese mice.

In the present study there was a significant reduction in protein profile in sucralose group but it was highly significant decrease in sodium saccharin group. **Hassan and Yousef[20]** announced an inhibitory effect of some food additives on the biosynthesis of protein and albumin, which indicated that the liver is unable to perform its functions. This may be resulted in a reduction of protein synthesis or especially albumin through the effect of sucralose or saccharin on the liver by inhibiting oxidative phosphorylation .

There was significant increase in ASAT and ALAT enzymes to the rats received sucralose , this may be due to hepatotoxicity and liver damage, as the more severe the liver damages the higher the release of the liver enzymes [21].

Also, there was a highly significant increase in ASAT and ALAT enzymes after using sodium saccharin. **Osfor & Elias[22]** reported that saccharin treated rats showed a significant increase in ALAT activity after both 6 and 12 weeks of administration. ASAT levels were significantly higher in rats received saccharine, where chronic saccharin intake reflects some abnormal changes in metabolic, hormonal and neural responses in males and female rats[23]. The elevation in serum aminotransferase activities could be due to severe effects caused by free radicals that interact with cellular membranes or related to breakdown of liver parenchyma. The changes in liver function could be due to hepatocellular impairment which subsequently caused leakage and release of greater than normal levels of intracellular enzymes into the blood[16].

The administration of sucralose reduces the level of triglycerides, while elevates the level of total cholesterol, HDL-C and LDL-C, when compared to control rats. The decrease of triglycerides might be attributed to the effect of sucralose on the peroxisome proliferator-activated receptors-alpha (PPAR-α) thus increasing the expression of lipoprotein lipase. In addition, activation of PPAR-γ in adipose tissue stimulates triglyceride storage[24]. The increase of HDL-C might result from the effect

of sucralose on PPAR-α and activation of apo A-I and apo A-II. The increased cholesterol following administration of sucralose may be attributed to increased intestinal absorption and/or increased cholesterol synthesis[25].

Our study revealed that saccharin induced hypocholesterolemia and hypotriglyceridemia when compared to control groups which was in agreement with the results recorded by **Ashour and Abdelaziz**[26]. The mechanism of hypocholesterolemic and hypolipidemic effect induced by saccharin may be due to the suppressive offect of saccharin on liver enzymatic activity of acetyl-CoA synthetase, citrate lyase, and mitochondrial citrate exchange leading to a reduction of available cytoplasmic acetyl-CoA, which is required for the synthesis of cholesterol and fatty acids[27]. Furthermore, liver acetyl-CoA carboxylase, phosphatidate phosphohydralase, and glycerol-3-phosphate acyl transferase activities were reduced by the saccharin analogues. Suppression of these enzymes would lead to a reduction of triglyceride synthesis. The saccharin analogues accelerated bile excretion of cholesterol metabolites and increased the fecal excretion of the cholesterol, triglycerides, neutral lipids, and phospholipids, thus, the liver and plasma lipoprotein lipid contents including, cholesterol, triglycerides, and neutral lipids were markedly reduced by the saccharin[5].

There was a significant increase in creatinine and urea in sucralose treated group, while it was highly significant increase in sodium saccharin group. This may be due to the toxic effects of these artificial sweeteners on the kidney that can lead to disorders in the renal function, due to a reduction in glomerular filtration rate followed by retention of urea and creatinine in the blood[28]. Also, there is a study of **Singh**[29] who reported the ability of saccharin to produce bladder cancer in two generation biassays.

By using sucralose , there was a decrease in thyroid hormones T3 and T4.

Study of **Goz´dzik *et al*.**[30] explained that sucralose intake seems to diminish thyroid axis activity by decreasing TPO activity, TSH, and plasma total TH concentrations, but at the same time, it increases both free T3 and T4 indexes. Those findings confirmed that sucralose is physiologically active and may stimulate disturbances in pituitary-thyroid axis activity, and also regular exposure to sucralose might alters the thyroid axis response to ingested food[31].

Also, in **"Very Well health" magazine**[32] there was a report about artificial sweeteners and their effect on thyroid activity: according to a report presented at the 2015 International Thyroid Congress[33], the use of artificial sweeteners may be linked to the development of a type of hypothyroidism called Hashimoto's thyroid disease (HT). The research, conducted by investigators in New York City, who had been positively diagnosed with (HT). The use of artificial sweeteners within this population—including aspartame (Equal, NutraSweet) and sucralose (Splenda)—correlated with elevated levels of thyroid stimulating hormone (TSH). Increased TSH levels are considered indicative of hypothyroidism. In the study, two of every three who had subsequently stopped using artificial sweeteners had a complete reversal of their HT. Their thyroid antibodies gradually returned to normal, and they were even able to stop their hormone replacement medication. This response to discontinuing the sweeteners supports the idea that artificial sweeteners may play a role in thyroid disease[32]. On the other hand, there was a significant increase in thyroid hormones when rats received sodium saccharin. These changes in thyroid hormones could also be attributed to the alteration in the pitutary – thyroid axis as a consequence of the stressing effect of sodium saccharin[34].

There was a significant decrease in testosterone hormone in rats received sodium saccharin. In mice, an earlier study discussed the effects of saccharin on the reproductive functions has shown that saccharin reduced fertility. CAMP and PKA are known to be key regulators of steroidogenesis in Leydig cells[35]. In Leydig cells, higher cAMP levels are essential to produce testosterone in response to LH. In the present study, the differential changes found in LH level after saccharin treatment suggest that there are pituitary effects altering LH, and these changes are related to different levels in testosterone production. Also, saccharin leads to attenuated sperm quality (including sperm count, viability, motility, and augmented abnormalities)[34].

CONCLUSION

We recommended to reduce or stop using artificial sweeteners and replace them with natural products. Other toxicological studies must be done on them.

REFERENCES

1. **Uçar A and Yilmaz S (2015):** Saccharin genotoxicity and carcinogenicity: a review. Advances in Food Sciences, 37:3.
2. **Harpaz D, Yeo LP, Cecchini F *et al.* (2018):** Measuring artificial sweeteners toxicity using a bioluminescent bacterial panel. Molecules, 23: 2454.
3. **Gardner E (2018):** Alternative sugars, sucralose. The magazine of UPFRONT, British Dental Journal, 224: 1.
4. **Saada HN, Mekky NH, Eldawy HA *et al.* (2013):** Biological effect of sucralose in diabetic rats. Food and Nutrition Sciences, 4: 82-89.

5. **Amin KA, Al-muzafar HM, Abd Elsttar AH (2016):** Effect of sweetener and flavoring agent on oxidative indices, liver and kidney function levels in rats. Indian Journal of Experimental Biology, 54:56-63.
6. **Gong T, Wei Q, Mao D *et al.* (2016):** Effects of daily exposure to saccharin and sucrose on testicular biologic functions in mice. Biology of Reproduction, 95(6):116, 1–13.
7. **Singh Z (2013):** Toxicological Aspects of Saccharin. Food Biology, 2(1):04-07.
8. **Friedewald WT, Levy RI, Fredrickson DS, *et al* (1972):** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin.Chem.,18:499-502.
9. **Norbert WT (1995):** Clinical guide to laboratory tests. 3rd ed. Saunders W. B., Company, Philadelphia.
10. **Rosales-Gómez CA, Martínez-Carrillo BE, Reséndiz-Albor AA *et al.* (2018):** Chronic Consumption of Sweeteners and Its Effect on Glycaemia, Cytokines, Hormones, and Lymphocytes of GALT in CD1 Mice. BioMed Research International, 2018:15.
11. **Schiffman SS and Rother KI (2013): Sucralose, a synthetic organochlorine sweetener: overview of biological issues**. Journal of Toxicology and Environmental Health, 16:399–451.
12. **Dib K, Oget I, Wrisez F *et al.* (1996)**:- Effects of sodium saccharin diet on fat-cell lipolysis: evidence for increased function of the adenylyl cyclase catalyst. Int. J. Obes. Relat. Metab. Disord., 20(1): 15-20.
13. **Abdallah** IZA (2002): Physiological changes induced by long term administration of saccharin compared with aspartame to male albino rats. The Egyptian Journal of Hospital Medicine, 8: 70-81.
14. **Abou-Donia MB, El-Masry EM, Abdel-Rahman AA *et al.* (2008):** Splenda al-ters gut microflora and increases intestinal p-glycopro- tein and cytochrome p-450 in male rats. Journal of Toxicology and Environmental Health, 71(21):1415-1429.
15. **Jacquillet G, Debnam ES, Unwin RJ *et al.* (2018):** Acute saccharin infusion has no effect on renal glucose handling in normal rats in vivo. Physiol Rep., 6 (14): e13804.
16. **Alsoufi MA, Aziz RA, Hussein ZG (2017):** Effect of some artificial sweeteners consumption in biochemical parameters of rats. Microbiology and Biotechnology, 5(3):1095-1099.
17. **Gupta S, Kalra S, Bharihoke V *et al.* (2014):** Sucralose induced pancreatic toxicity in albino rats: histomorphological evidence. J.Morphol. Sci., 31(2):123-127.
18. **Amornpan Lertrit MD, Sasinee Srimachai MD, Sunee Saetung MSc *et al.* (2018):** Effects of sucralose on insulin and glucagon-like peptide-1 secretion in healthy subjects: A randomized, double-blind, placebo-controlled trial. Nutrition, 55-56:125–130.
19. **BaileyCJ, Day C, Knapper JM, *et al.* (1997)**: Antihyperglycemic effect of saccharin in diabetic ob/ob mice. Br. J. Pharmacol., 120(1): 74-78.
20. **Hassan HA and Yousef MI (2010):** Ameliorating effect on chicory (*Cichorium intybus L.*)-supplemented diet against nitrosamine precursors-induced liver injury and oxidative stress in male rats. Food Chem. Toxicol., 48 (8–9): 2163–2169.
21. **Helal EGE, El-Sayed RAA, Hedeab GM *et al.* (2017):** The therapeutic effects of stem cell enhancer on changes of some physiological parameters in male albino rats treated with mixture of food additives) food preservative, food coloring agent, and flavor enhancer). The Egyptian Journal of Hospital Medicine, 67 (2): 591- 598.
22. **Osfor MMH and Elias TR (2003):** Nutritional and biochemical studies on some artificial sweeteners administrated to male albino rats. Bull Nat Res Centre, 28: 377.
23. **Andrejić BM, Mijatović VM, Samojlik IN *et al.* (2013):** The influence of chronic intake of saccharin on rat hepatic and pancreatic function and morphology: gender differences. Bosn J Basic Med Sci., 13: 94.
24. **Ferré P (2004):**The biology of peroxisome proliferator-activated receptors. Relationship with lipid metabolism and insulin sensitivity. Diabetes,53(1): 43-50.
25. **Kahnamoei JR and Ghaleh HA (2016):** The effect of commercial sweetener "cipla" on the serum lipid profiles in diabetes-induced rats. Crescent Journal of Medical and Biological Sciences, 3(4): 136–138.
26. **Ashour AA and Abdelaziz I (2009):** Role of fast green on the blood of rats and the therapeutic action of vitamins C or E. International Journal Integrative Biology, 6: 6.
27. **Hall IH, Voorstad PJ, Cocolas GH (1983):** Antihy-perlipidemic activity of saccharin analogues in rodents. J Pharm Sci.,72: 1192-8.
28. **Turley SD and Dietschy JM (2003):** Sterol absorption by the small intestine. Curr Opin Lipidol., 14: 233.
29. **Singh Z (2013):** Toxicological aspects of saccharin. Food Biology, 2(1):04-07.
30. **Goz´dzik** EP, **Bigos** A, **Huszcz** DR (2018): Type of sweet flavour carrier affects thyroid axis activity in male rats. Eur J Nutr., 57:773–782**.**
31. **Warner MH and Beckett GJ (2010):** Mechanisms behind the nonthyroidal illness syndrome: an update. J Endocrinol., 205:1–13.
32. **https://www.verywellhealth.com/thyroid-patients-should-avoid-artificial-sweeteners-3233020**
33. **https://www.liebertpub.com/doi/full/10.1089/thy.2015.29006.abstracts**
34. **Gong T, Wei QW, Mao DG et al. (2016):** Effects of Daily Exposure to Saccharin and Sucrose on Testicular Biologic Functions in Mice. Biology of Reproduction, 95(6):116, 1–13.
35. **Abdou HS, Bergeron F, Tremblay JJ (2014):** A cell-autonomous molecular cascade initiated by AMP-activated protein kinase represses steroidogenesis. Mol Cell Biol., 34:4257–4271.